*J. Exp. Biol.* (1967), 47, 191–200
*With 4 text-figures*
*Printed in Great Britain*



# HYPERPHAGIA IN THE BLOWFLY*

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(*Received* 3 *April* 1967)

The current view of the control feeding in the blowfly *Phormia regina* Meigen holds that ingestion is initiated by stimulation of oral taste receptors and ultimately terminated by signals from the foregut (Dethier & Bodenstein, 1958). The signals from the foregut originate in stretch receptors which monitor the passage of food from the crop to the mid-gut (Gelperin, 1966*b*). The recurrent nerve conveys these signals to the brain where they counteract input from peripheral taste receptors. Transection of the recurrent nerve interferes with this inhibitory feed-back system and causes a fly to become hyperphagic (Dethier & Bodenstein, 1958).

This proposed mechanism satisfied all the known facts concerning actual feeding behaviour. Nuñez (1964) has now demonstrated, however, that a related fly, *Lucilia* sp., becomes hyperphagic if the ventral nerve cord is cut between the brain and the thoracic ganglionic mass. It is obvious, therefore, that the control of feeding is not so simple as the foregoing hypothesis proposed. For this reason inquiry into the subject has been re-opened. At the same time advantage has been taken of newly developed techniques to refute some criticisms of Evans & Barton Browne (1960).

## MATERIALS AND METHODS

The flies were taken from the same stock as those employed in the original 1958 study. The following surgical operations were performed: (1) transection of the recurrent nerve posterior to the brain; (2) transection of the recurrent nerve anterior to the brain; (3) transection of the ventral nerve cord posterior to the suboesophageal ganglion; (4) transection of all abdominal nerves associated directly with the ventral cord; (5) amputation of all legs.

(1) Transection of the recurrent nerve posterior to the brain followed exactly the technique of Dethier & Bodenstein (1958).

(2) The anterior cut was made as follows. The fly to be operated upon was stood vertically on its tail in a depression in wax molded to its contours. In this position the front (frons and clypeus) of the head lies in a horizontal plane flush with the surface of the wax block. With a microscapel cuts were made along the two frontal sutures producing a single V-shaped incision. The apex of the cuticular flap was grasped by forceps and folded ventrally along a natural hinge formed by the epistomal suture. The flap was anchored in this position by a pin thrust across it and into the wax.

* This work was supported by grant GB 1472 from the National Science Foundation and United States Public Health Service Training Grant 5T1 GM281.
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Internal pressures exerted by opening the flap forced into view the loop of oesophagus that normally lies between the two furcal prongs when the proboscis is in the retracted position. A certain amount of fat-body overlies the oesophagus. When this is removed, the frontal ganglion can be seen lying dorsal to the oesophagus. Also visible are the connectives from the brain to the frontal ganglion and the recurrent nerve extending back into the posterior regions of the body. In one varient of this operation the recurrent nerve itself was transected; in another, the connectives to the ganglion were cut; in another all three were cut and the ganglion removed. The effect on subsequent events was the same in all cases. After the nerves had been cut the flap of cuticle was released. It fell naturally back into place. In order to make it secure, its apex was tucked under the adjoining cuticle. Blood clots sealed the wound.

(3) Transection of the ventral nerve cord was made through a simple incision on the ventral surface of the neck.

(4) To sever the nerves connecting the thoracic ganglion and abdomen, the fly was affixed ventral side up and all legs secured by strips of plasticene. The intersegmental membranes on the lateral and anterior edges of the first abdominal sternite were cut and the sternite was folded posteriorly. The field thus exposed contained the abdominal tracheal sacs laterally, with the crop duct and nerve connexions between thorax and abdomen situated medially. These nerves were cut and the cuticular flap was replaced.

(5) Legs were amputated at the coxotrochanteral joint.

Normal and experimental flies were treated in a variety of ways. In some cases food was administered by mouth by means of a microlitre syringe which permitted direct measurement of the quantity ingested at the time of feeding. During feeding the flies were held by the wings with clamp forceps. Thus they could be released for subsequent observation. In other cases flies were allowed to feed *ad lib.* by being confined in Petri dishes lined with filter-paper soaked in appropriate solutions. All flies were 2–4 days old. All had been given access to 0·1 M sucrose for 24 hr. and then deprived of all solutions for 24 hr.

## INDUCTION OF HYPERPHAGIA

*Criterion of hyperphagia.* The amount of sugar solution imbibed by a fly depends upon the previous nutritional history, the degree of activity during the preceding 24 hr., the weight of the fly, and the ambient temperature and humidity. Within any given sample of flies the standard deviation of the mean intake can vary from 7 to 19% of the mean. There is also significant inter-sample variability from one day to the next; the standard deviation of the sample means in this study was 13% of the average sample mean. Because of these levels of variability an absolute quantitative criterion of hyperphagia is unrealistic.

A fly was considered to be hyperphagic when it ingested not less than twice the quantity of fluid taken by a control from the same sample. For purposes of summary, however, the data in Table 1 represent consolidations of all samples receiving similar treatments. It is clear from an examination of this table that several different kinds of treatment cause a fly to become hyperphagic.

*Transection of the recurrent nerve.* As Dethier & Bodenstein (1958) reported, and

Green (1964) confirmed, transection of the recurrent nerve posterior to the brain causes hyperphagia. At the site of the operation the recurrent nerve lies close to the endocrine complex, the corpus allatum and the corpora cardiaca, and contains fibres connecting these glands with the brain. Evans & Barton Browne (1960) questioned the conclusion that hyperphagia following recurrent nerve transection was in fact a consequence of interrupting neural transmission. They asserted that their own operations resulted in hyperphagia only 31% of the time and that the 'neatest' operations did not necessarily produce hyperphagia. No confirmatory autopsies were reported. Green (1964), on the other hand, in a meticulous study confirmed by post-mortem examination, produced hyperphagia in 69% of the cases in which the recurrent nerve was cut with care and precision. We have since carried out more than 200 recurrent nerve operations and fully confirm Green's conclusions.

Table 1. *Effect of cutting the ventral nerve cord (VNC), the recurrent nerve (RN), the frontal ganglion (FG), and the legs on the volume of* 2·0 M *glucose ingested by flies standing on sugar-saturated filter-paper*

| Operation | Mean intake (μl.) | Standard deviation | *n* |
|---|---|---|---|
| Sham VNC/RN | 15·0 | 5·5 | 28 |
| RN | 37·4 | 6·4 | 20 |
| VNC | 39·4 | 7·3 | 31 |
| Sham FG | 18·2 | 3·1 | 8 |
| FG | 37·8 | 9·4 | 20 |
| Legless | 31·3 | 9·7 | 9 |

The fact still remains, however, that the recurrent nerve posterior to the brain is a mixed nerve including among its fibres the nervi corporis cardiaci I which afford neural connexion between the neurosecretory cells of the pars intercerebralis and the corpora cardiaca and corpus allatum. Thus, when a posterior cut is made, it severs these connexions as well as isolating the nerves of the gut. To circumvent these complications the technique of transecting the nerve anterior to the brain was developed. The fibres from the endocrine glands travel in the recurrent nerve only as far as the posterior edge of the brain at which point they leave to make independent connexions. In the region of the frontal ganglion the recurrent nerve has no associations with the endocrine complex. Transections at this point resulted in hyperphagia indistinguishable from that produced by posterior transection. In Table 1 the posterior transection is designated as RN, the anterior section in the region of the frontal ganglion, as FG.

We can reaffirm, therefore, that the recurrent nerve is directly concerned with the regulation of feeding and that interrupting its transmission from the foregut to the brain causes hyperphagia.

*Transection of the ventral nerve cord.* The report of Nuñez (1964) that transection of the ventral nerve cord results in hyperphagia has been amply confirmed. Every one of thirty-one flies in which this operation was performed became hyperphagic. In Table 1 this operation is designated as VNC.

Cutting the ventral cord deprives the brain of all sensory input from the thorax and abdomen and silences many potential sources of information relative to feeding.

Most particularly the operation isolates tactile and proprioceptors in the body-wall and the tarsal chemoreceptors. Furthermore, none of the activity generated in the ventral ganglionic mass, including thoracic locomotor centres, reaches the brain. An operated fly is unable to fly or walk, but it can stand unsteadily. It is perfectly capable of extending and retracting the proboscis; however, even though the fly is placed on a paper saturated with sugar, the proboscis remains in the retracted position because tarsal input does not reach the brain. Hyperphagia occurs only if the labellum inadvertently encounters the substrate as a consequence of postural instability. If a fly maintains a standing posture such that the labellum does not touch the substrate, there is no hyperphagia. The absence of spontaneous proboscis extension argues against the proposition that hyperphagia arises as a consequence of some endogenous brain centre being released from inhibitory control of the thoracic ganglion.

*Transection of body-wall nerves.* When a fly ingests a full meal, the expanded crop fills most of the abdominal cavity. Pressure from the crop is transmitted to the body-wall. It is conceivable that there are receptors associated with the body-wall which respond to this force. These receptors would have axons terminating in the thoracic ganglion. Cutting the nerves connecting the abdomen and the thoracic ganglion by the technique previously described resulted in vigorous hyperphagia.

*Hand-feeding and leg amputation.* The foregoing experiments involving denervation presumably induce hyperphagia by interfering with internal sources of negative feedback in the homeostatic mechanism underlying feeding. Observations made during the course of these experiments suggested that the same end result—hyperphagia—might be produced by a generically different approach: continuous stimulation of the labellar receptors. This was accomplished in two ways. If flies are hand-fed in such a way that the drop of sugar solution is continuously applied to the labellar lobes, even when the labellum is retracted, the fly feeds intermittently and gradually becomes hyperphagic. If the legs are amputated at the coxotrochanteral joint and the hexaplegic fly placed on a disk of filter-paper saturated with 2·0 M glucose, the labellum is unavoidably stimulated a majority of the time. These flies also become hyperphagic.

## PATTERNS OF FEEDING

There appeared to be no way to reconcile the foregoing diverse results when the sole criterion of hyperphagia was the quantity of fluid ingested. Accordingly, the temporal pattern of feeding was examined over an extended period. The incidence and duration of proboscis extension were recorded by an observer employing an event-recorder. Results typifying the observations are illustrated in Figs. 1 and 2 where patterns of response of four flies selected from a single sample are compared.

A normal, or sham-operated, unrestrained fly, deprived of food for 24 hr., imbibes from 6·5 to 24·5 $\mu$l. of 2 M glucose during its first meal. A meal is one uninterrupted bout of sucking. The sham-operated fly whose activity is diagrammed in Fig. 1 took a first meal of 99 sec. duration whereupon the proboscis was retracted. This meal was followed by a series of short drinks over the next 10 min. and then no further drinking during the next 30 min. The total duration of drinking within the 30 min. period was 106·0 sec.; the total amount imbibed, 28·7 $\mu$l.; the rate of sucking, 0·27 $\mu$l./ sec.

The fly lacking its recurrent nerve connexion (RN) took an initial meal lasting

116 sec. (Fig. 1). Over the next 30 min. it took seven drinks of 10 or more seconds duration. The total duration of drinking in the 30 min. period was 116 sec.; the total amount imbibed, 46·0 $\mu$l.; the rate of imbibition, 0·19 $\mu$l./sec.

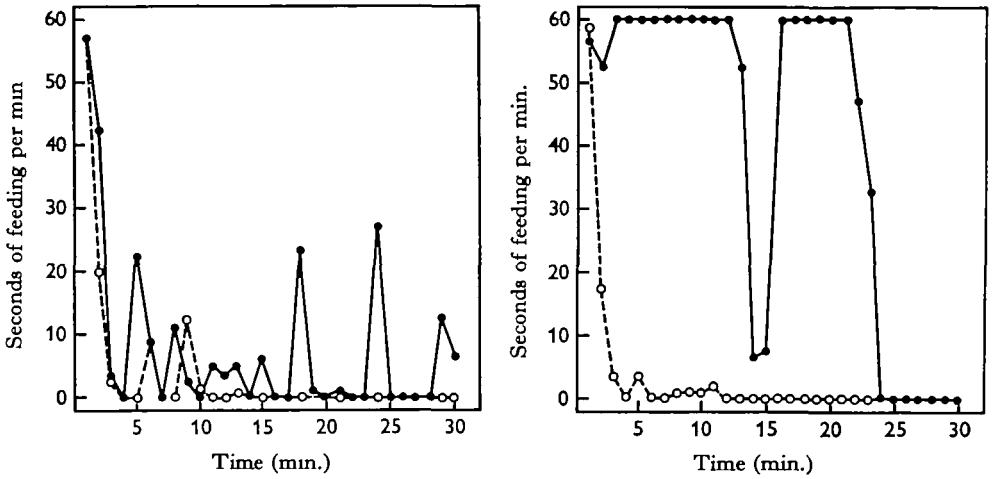


Fig. 1. Comparison of the pattern of feeding of a fly with the recurrent nerve cut (RN) (solid circles) and a sham-operated (open circles) control during a 30 min. exposure of 2 M glucose.
Fig. 2. Comparison of the feeding pattern of a fly with the ventral nerve cord cut (VNC) (solid circles) and a sham-operated control (open circles) during a 30 min. exposure to 2 M glucose.

The fly with the interrupted ventral nerve cord (VNC) took essentially one meal (Fig. 2) which resulted in bursting after 24 min. The total intake just prior to bursting was 42·0 $\mu$l. The sham-operated control followed the usual normal pattern of ingestion resulting in a total intake of 18·1 $\mu$l. during the 30 min. exposure to 2 M glucose. Flies which had only the nerves to the abdomen cut took an initial meal lasting four times longer than that of controls. They then began to walk about. If the ventral nerve cord was then cut between the brain and thoracic ganglionic mass, the labellum was touched to the substrate and feeding recommenced and lasted until bursting.

The patterns of feeding in hand-fed and legless flies are in fact determined by the experimenter since proboscis extension occurred only when the labellum came into contact with the solution. When the experimenter attempted to maintain continuous contact, an indication of pattern emerged. The initial meal was normal. The pattern of subsequent bouts resembled that characteristic of the RN fly. The end result was hyperphagia.

When the amount of fluid imbibed by each kind of fly was measured at intervals over an 8 hr. period, some additional differences were revealed (Fig. 3). At the end of the first 30 min. hyperphagia had already been established. This finding is in accord with the results reported above. From 30 min. to 8 hr. all three kinds of flies decreased their intake and reached an asymptote, but the rates of decline differed. The normal fly reached final capacity most rapidly. Lack of further increase resulted from lack of ingestion. The VNC fly had reached nearly maximum capacity within the first 30 min. Thereafter, there was only a small increase in volume (as was true of the normal fly); however, failure to increase further resulted not from lack of ingestion but from inability to force more fluid into the gut. The RN fly, on the other

hand, while clearly hyperphagic at the end of 30 min. had not attained the same level of hyperphagia as the VNC fly. It then continued to increase its weight gradually.

In short, as all of these pattern analyses reveal, there is more than one kind of experimental hyperphagia in flies.

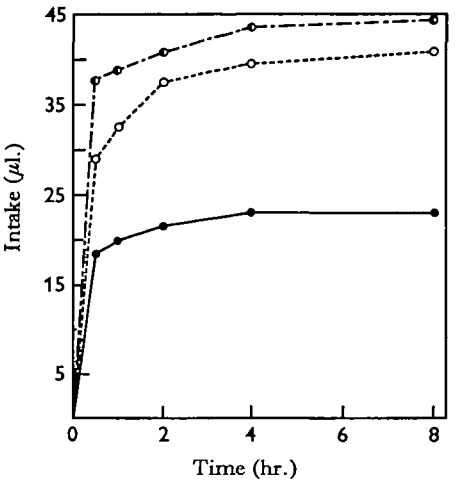


Fig. 3. Comparison of the volume of 1 M fructose ingested by a fly with the recurrent nerve cut (RN) (dotted line), one with the ventral nerve cord cut (VNC) (broken line), and a control (solid line) over an 8 hr. period.

## DISCUSSION

The foregoing results indicate that the control of ingestion is more complicated than originally believed. The fact remains, however, that feeding is very much under the control of sensory input from peripheral chemoreceptors and of post-ingestive inhibitory feedback. The original hypothesis of Dethier & Bodenstein (1958) proposed that a receptor in the region of the foregut, which monitored the transfer of fluid from crop to mid-gut and delivered to the brain impulses that had the effect of nullifying sensory input, caused feeding to stop. There is considerable experimental evidence favouring the existence of such a receptor (Dethier & Bodenstein, 1958; Gelperin, 1966*a*).

The strongest single piece of evidence is the occurrence of hyperphagia following transection of the recurrent nerve. Evans & Barton Browne (1960) had cautioned that surgical interference with the recurrent nerve might equally well affect neural and hormonal events since at the point of transection the nerve carried some fibres from the endocrine complex. This interpretation is negated by the finding that transection anterior to the brain has precisely the same effect as posterior transection in the region of the endocrine glands. There is very little doubt that direct neural monitoring of post-ingestive events in the foregut plays a prominent role in the termination of ingestion. Additional work by Gelperin (1966*b*) indicates that the monitor is in all probability a stretch receptor.

Other post-ingestive events also have a part in terminating feeding. Their importance is demonstrated by transecting the ventral nerve cord posterior to the brain. As Nuñez (1964) first showed, flies subjected to this operation become hyperphagic even though the recurrent nerve is still intact.

Cutting the ventral nerve cord obviously deprives the fly of multiple sources of information: tarsal chemoreceptors, abdominal stretch and proprioceptors, and any activity that may be generated in locomotor or other centres in the thoracic ganglionic mass. The fact that cutting only the nerves to the abdominal body-wall induces hyperphagia suggests that normal termination of ingestion is mediated in part by body-wall receptors. The fact that this hyperphagia is somewhat different in pattern from that induced by total cord section suggests that elimination of the locomotor centre and the tarsal receptors is also a significant factor.

It is clear that the process of feeding is regulated by the interplay of varying excitatory input from chemoreceptors and fluctuating inhibitory feedback from mechanoreceptors in the gut and body-wall. Imbalance in one direction initiates and drives feeding; imbalance in the opposite direction terminates it. Neither of the two feedback mechanisms can operate effectively in the absence of the other. Surgical interference with either induces hyperphagia, but the patterns of hyperphagia are not identical. By comparing the difference it is possible to arrive at some understanding of the nature of the total integrated mechanism controlling feeding.

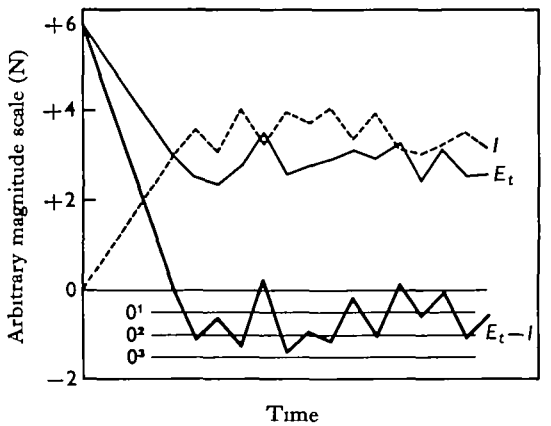


Fig. 4. Model proposed to explain the phenomenon of hyperphagia and the interaction of excitatory and inhibitory factors regulating feeding behaviour in the normal fly. Consult text for full explanation.

The model (Fig. 4) which is proposed to unify all of the data has as its central postulate that the occurrence of the behaviour being studied is the result of an interplay between excitation and inhibition. Since no evidence for an endogenous neural centre driving feeding behaviour has been found, it appears that the sole source of excitatory input which initiates and drives feeding behaviour is the peripheral chemoreceptors on tarsi and labellum. It must be noted that the excitatory inputs from tarsi and labellum initiate separate bits of behaviour—tarsal excitation initiates proboscis extension; labellar excitation initiates spreading of the oral lobes and drives sucking. Tarsal excitation makes only a negligible contribution to the act of sucking. Neural activity that inhibits feeding response arises from three sources: (1) stretch receptors in the foregut send information via the recurrent nerve to monitor the rate and extent of peristalsis in the foregut ($I_{fg}$); (2) stretch receptors in the body-wall send

information via the thoracic ganglion and ventral nerve cord to monitor the distension caused by the fullness of the crop ($I_{bw}$); (3) a locomotor centre in the thoracic ganglion produces activity which is inhibitory to feeding behaviour ($I_{lc}$). If total excitation is represented by $E$ and total inhibition by $I$, then the central postulate of the model states that feeding behaviour is absent when $E-I \leqslant 0$; when $E-I > 0$, feeding behaviour is initiated. When the proboscis is retracted, $E = E_{\text{tarsal}}$, when the proboscis is extended, $E = E_{\text{labellar}}$; the same inhibition interacts with both $E_t$ and $E_l$.

Consider a food-deprived fly walking on a disk of filter-paper saturated with 1·0 M sucrose. At first contact, $E_t$ will be high because the tarsal receptors are completely disadapted and the stimulus is intense; $I$ will be low because the gut is empty. This is the state of events at time 0 on Fig. 4. As the fly feeds, its tarsal receptors are adapting, and internal inhibition is increasing as the gut is filled. Feeding is terminated as a result of the build-up of inhibition and the adaptation of the *labellar* receptors. The tarsal receptors are now at a steady-state level of adaptation; however, as the fly walks in the sugar field new receptors are stimulated and adapted producing fluctuation in the amount of excitatory tarsal input. Similarly, fluctuations in the inhibitory input are caused by the discontinuous nature of foregut peristalsis and the churning of the crop in the abdomen. The values of both $E$ and $I$ oscillate randomly about their mean values. Consequently, the values of $(E-I)$ also fluctuates, aperiodically becoming greater than zero and initiating a meal. It should be noted that the meal is initiated by $E_t-I$ becoming greater than zero, while the duration of the meal is determined by the length of time that $E_t-I$ is greater than zero. In Fig. 4 a proboscis extension is initiated whenever the $E_t-I$ line intersects the 0 line with a positive slope, i.e., from underneath.

To determine the effect of cutting the recurrent nerve, the ventral nerve cord, or the posterior connectives, consider the effect of lowering the $I$ curve by a given amount at every point along its length. Lowering the $I$ curve is equivalent graphically to raising the $E-I$ curve; raising the $E-I$ curve is equivalent graphically to lowering the zero line. Therefore to determine the effect of removing a source of inhibition, for example, removing $I_{fg}$ by cutting the recurrent nerve, the zero line is lowered by an amount equal to $I_{fg}$ (for example, to $0^1$). The $E_t-I$ line now becomes positive a greater number of times; hence more meals are taken. This is the experimental observation. If a larger source of inhibition is removed, say $I_{bw}$, then the situation is analysed graphically by lowering the 0 line further, to $0^3$ for example. Again the prediction of more meals per unit time is the correct one. If we assume that $E_l$ is proportional to $E_t$, then the length of the meal is proportional to the length of time that the $E_t-I$ line is above the 0 line; this is also seen to increase as sources of inhibition are removed and the 0 line is lowered. Finally, if sufficient sources of inhibition are removed, for example by removing $I_{lg}$ and $I_{bw}$, continuous feeding results because $E-I$ is continually greater than zero. Cutting the ventral nerve cord produces this effect, predicted graphically by lowering the 0 line to $0^3$.

Evidence for the foregoing hypothesis of feeding regulation in the blowfly can be summarized as follows:

*Contribution of adaptation to the termination of feeding.* Feeding can be driven by stimulating one half of the labellum; when this becomes ineffective feeding can be

restarted by stimulating the other side (Dethier, Evans & Rhoades, 1956). When feeding ceases, it can be restarted by stimulating with a higher concentration (Dethier *et al.* 1956). Electrophysiological recording shows that receptor adaptation does indeed occur. The labellar hairs adapt within 5–20 sec. but seldom reach zero level of firing. The oral papillae maintain a steady state of firing in the adapted state for many minutes (Dethier & Hanson, 1965). Some tarsal receptors also continue to fire at a low level for as long as 15 min., although as a general rule tarsal adaptation is more rapid than labellar and usually reaches zero level of firing.

*Recurrent nerve involvement.* Transection produces hyperphagia. Transection anterior to the brain is as effective as posterior transection; hence, the endocrine complex is not involved. Flies lacking recurrent nerve connexions periodically extend their proboscis when they are standing in a sugar-soaked substrate whereas normal flies do not. In other words, inhibition from the foregut receptor transmitted via the recurrent nerve normally interacts with input from tarsal receptors. After feeding, acceptance thresholds to sugar applied to tarsal receptors do not rise as high or as rapidly in operated animals as they do in sham-operated controls. Flies with the recurrent nerve cut do not react abnormally to water as Evans & Barton Browne (1960) maintained.

*Ventral nerve cord involvement.* Transection produces hyperphagia. Transection effectively removes tarsal input and hence the control of the recurrent nerve on tarsal threshold. Transection of nerves to body wall removes input from receptors recording stretch. In the absence of the ventral cord adaptation of the oral receptors cannot shut off feeding even though the recurrent nerve is intact; therefore, the ventral cord applies more central inhibition to oral input than does the recurrent nerve.

*Absence of ganglionic centres driving feeding.* Flies with either or both the recurrent nerve and/or ventral cord cut will not extend the labella spontaneously. Stimulation of labellar and oral receptors by sugar is required to initiate and drive feeding. Substitution of water for sugar during a meal terminates sucking in normal, RN, and VNC flies. Cutting nerves to the body-wall and leaving the abdominal ganglion intact has the same effect as destroying the ganglion. Flies do not walk and feed simultaneously so it appears that locomotion exerts an inhibitory effect on feeding.

## SUMMARY

1. The nervous mechanism controlling feeding in the blowfly has been re-investigated.

2. The data presented reveal that the mechanism is more complex than originally thought but can be readily understood in the same basic terms as the original model, that is, the interaction of peripheral sensory excitation and internal inhibition.

3. The old model becomes the new by the addition of two new sources of internal inhibition—body-wall stretch receptors and a thoracic ganglion locomotor centre.

4. The several sources of internal inhibition are not equal in their inhibitory effect; removing body-wall stretch receptors produces a more vigorous hyperphagia than removing the foregut stretch receptor.

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